DP-9024, an investigational small molecule modulator of the integrated stress response kinase PERK, causes B-cell cancer growth inhibition as single agent and in combination with standard-of-care agents

Gada Al-Ani, Qi Groer, Aaron J Rudeen, Kristin M Elliott, Patrick C Kearney, Jeffery D Zwicker, Yu Mi Ahn, Stacie L Bulfer, Cale L Heiniger, Molly M Hood, Salim Javed, Joshua W Large, Max D Petty, Kristen L Stoltz, Bertrand Le Bourdonnec, Bryan D Smith, and Daniel L Flynn

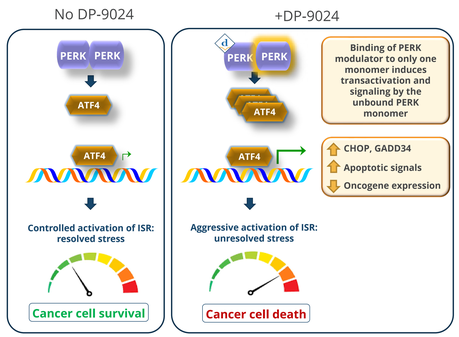
Deciphera Pharmaceuticals, LLC, Waltham, MA, USA

decīphera®

Poster: 1640

Introduction

- The Integrated Stress Response (ISR) is a major adaptive stress response pathway in cancer cell maintenance[1-4]
- The ISR family member PERK is a member of the Unfolded Protein Response (UPR) pathway which plays a role in resolving endoplasmic reticulum (ER) stress such as processing unfolded proteins[1-4]
- The UPR is considered an Achilles' heel in B-cell cancers, as multiple myeloma and B-cell lymphoma are dependent on a well-balanced UPR pathway to cope with the high demand for protein folding and their secretory nature
- Given the double-edged–sword nature of the UPR, the activation of PERK and its downstream pathway can have cytoprotective or cytotoxic effects[5]
- In B-cell cancers, the UPR is at close-to-maximum cytoprotective capacity, such that further pharmacological stimulation of PERK can potentially be leveraged to cause a cancer cell cytotoxic response and induce antitumoral effects



Methods

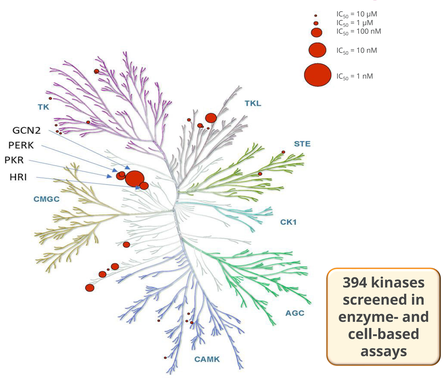
- Modulation of ISR kinases was characterized using enzymatic assays
- Kinome selectivity profiling was determined using enzymatic and cellular assays
- Cellular modulation of the ISR/UPR pathway (PERK, ATF4, and CHOP) or the apoptosis pathway (c-PARP, c-Caspase 3/7) was measured by Western blot, qRT-PCR, or ELISA
- *In vivo* upregulation of tumoral ATF4 was determined in a MM pharmacokinetic/pharmacodynamic xenograft model
- *In vivo* inhibition of tumor growth was determined in MM and B-cell lymphoma xenografts

Results

DP-9024 was designed as a selective and potent modulator of ISR kinases that activates PERK, with optimized pharmaceutical and selectivity profiles

	Assay	DP-9024
Cellular assays	PERK dimerization assay (BRET; EC_{50}, nM)	274
	H929 ATF4 stimulation (fold increase versus control)	12
Off-target profile	Kinome and safety	Highly selective
	hERG (Predictor™ fluorescence polarization; IC_{20}, μM)	>20
ADME	Microsomal stability (human, mouse) % remaining at 60 min	64%, 70%
	Caco-2 (A-B, efflux ratio)	41, 1.6

DP-9024 exhibits a selective kinome profile



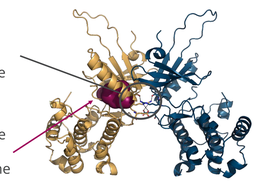
394 kinases screened in enzyme- and cell-based assays

Illustration reproduced courtesy of Cell Signaling Technology, Inc.

Structure of close DP-9024 analog bound to PERK monomer

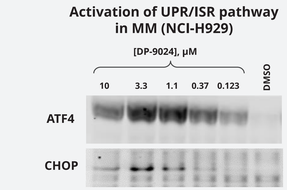
PERK forms a head-to-head dimer nucleated by trans-monomer hydrogen bond interactions with key arginine residues in the **C-helix switch**

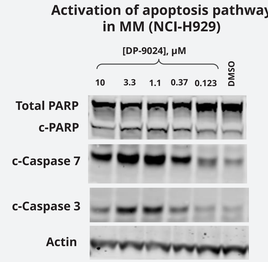
Binding **C-helix switch** in the ***"C-helix out"*** state limits inhibitor binding to half of the dimer, **transactivating** the unbound PERK half of the dimer in the catalytically active, ***"C-helix in"*** state

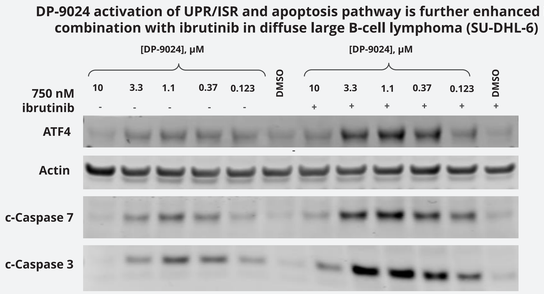


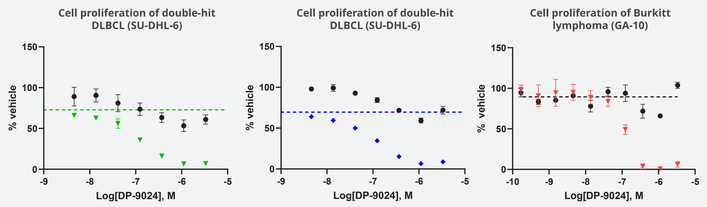
Both monomers were bound to compound under the crystal structure–saturating conditions. In this figure, only 1 bound monomer is shown to represent model of activation at or below IC_{50} of DP-9024 binding.

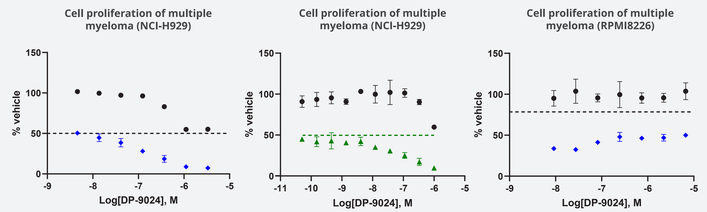
PERK activator DP-9024 activates the ISR pathway, induces apoptosis, and inhibits B-cell proliferation in combination with clinical agents



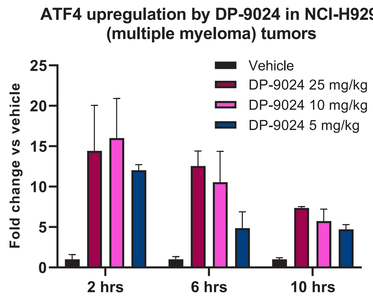


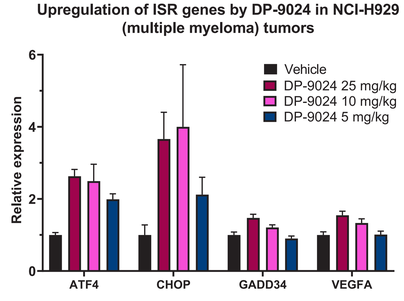




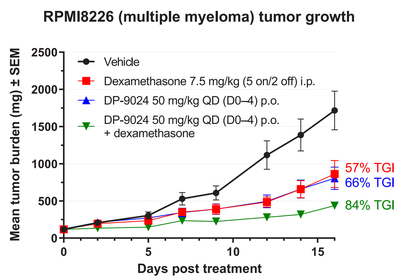


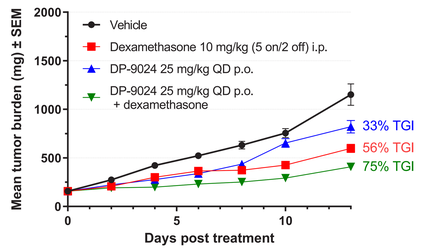
DP-9024 upregulates tumoral ISR and inhibits B-cell cancer tumor growth in combination with anti-myeloma agents in mouse xenograft models *in vivo*

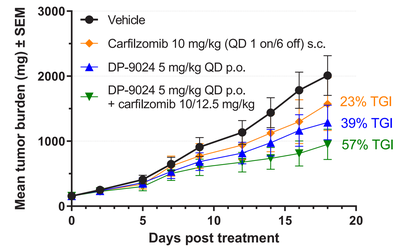




Dose (mg/kg)	Timepoint (hrs)	DP-9024 free plasma levels (ng/mL)	Fold ATF4 upregulation
25	2	99	14
25	6	104	13
25	10	116	7
10	2	70	16
10	6	47	11
10	10	59	6
5	2	42	12
5	6	38	5
5	10	48	5







CONCLUSIONS

- The ISR/UPR is a targetable vulnerability in cancers with high basal levels of ER stress
- DP-9024 increases UPR signaling by directly binding to one PERK monomer and inducing dimerization and transactivation of the unbound PERK monomer
- This novel mechanism leads to antitumoral effects in B-cell cancers *in vitro* and *in vivo*, likely through the induction of unresolved UPR stress, which may provide an alternative mechanism to current UPR-targeting therapies

PRESENTED AT THE AMERICAN ASSOCIATION FOR CANCER RESEARCH (AACR) ANNUAL MEETING
ORLANDO, FL, APRIL 14–19, 2023

CORRESPONDING AUTHOR/DISCLOSURES

Gada Al-Ani (Galani@Deciphera.com)
All authors are/were full time employees of Deciphera Pharmaceuticals, LLC and own/owned Deciphera Pharmaceuticals, LLC stock or options.

ACKNOWLEDGMENTS

Greg Plowman, Fred Reu, Cheryl Gradziel, Carla Marashio, Alex Thibonnier, Sihyung Yang, Dan Tanner, Forrest Stanley.
Editorial support was provided by AlphaBioCom, a Red Nucleus company, and was funded by Deciphera Pharmaceuticals, LLC.

ABBREVIATIONS

ADME, absorption, distribution, metabolism, and excretion; ADP, adenosine diphosphate; AGC, protein kinase A, G, and C families; ATF4, activating transcription factor 4; BRET, bioluminescence resonance energy transfer; c-, cleaved; CHOP, C/EBP homologous protein; CK1, casein kinase 1 family; CMGC, family of kinases including cyclin-dependent kinases, mitogen-activated protein kinases, glycogen synthase kinases, and cyclin-dependent kinases; DLBCL, diffuse large B-cell lymphoma; DMSO, dimethyl sulfoxide; EC_{50}, half maximal effective concentration; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; GADD34, growth arrest and DNA damage-inducible protein 34; GCN2, general control nonderepressible 2; hERG, human ether-a-go-go–related gene; HRI, heme-regulated inhibitor; IC_{20}, concentration inducing 20% inhibition; IC_{50}, half maximal inhibitory concentration; i.p., intraperitoneally; ISR, integrated stress response; MM, multiple myeloma; PARP, poly ADP-ribose polymerase; PERK, protein kinase R-like endoplasmic reticulum kinase; PKR, protein kinase R; p.o., orally; QD, once daily; qRT-PCR, real-time quantitative reverse-transcription polymerase chain reaction; s.c., subcutaneous; SEM, standard error of the mean; STE, homologs of yeast sterile 7, sterile 11, and sterile 20 kinase family; TGI, tumor growth inhibition; TK, tyrosine kinase family; TKL, tyrosine kinase–like family; UPR, unfolded protein response; VEGFA, vascular endothelial growth factor A.

REFERENCES

1. Marciniak SJ, et al. *Nat Rev Drug Discov.* 2022;21(2):115-40.
2. Pakos-Zebrucka K, et al. *EMBO Rep.* 2016;17(10):1374-95.
3. Licari E, et al. *Int J Biochem Cell Biol.* 2021;139:106059.
4. White-Gilbertson S, et al. *Front Genet.* 2013;4:109.
5. Vincenz L, et al. *Mol Cancer Ther.* 2013;12(6):831-43.